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Conservation planning for adaptive and neutral evolutionary processes

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Abstract

1. Protected area systems should ideally maintain adaptive and neutral evolutionary processes. To achieve this, plans for expanding protected area systems (prioritizations) can improve coverage of related attributes (evolutionary attributes). However, long-standing challenges in mapping and operationalizing evolutionary attributes have prevented their widespread usage.

2. We outline a novel framework for incorporating evolutionary processes into conservation planning. Using three amphibian species in the Iberian Peninsula (*Hyla molleri*, *Pelobates cultripes* and *Rana iberica*), we mapped a comprehensive range of adaptive and neutral evolutionary attributes to delineate places containing individuals with moderate to high heterozygosity, different neutral genetic clusters, different adaptive genetic clusters and climatic refugia. We overlaid these maps with boundaries of existing protected areas to quantify representation shortfalls and generated a prioritization to identify additional priority areas. To assess the performance of conventional approaches, we also generated a prioritization using only the species' distribution data—without the evolutionary attributes.

3. We found that existing protected areas within the Iberian Peninsula are failing to adequately represent evolutionary attributes for the study species. Specifically, they are not adequately representing places predicted to contain individuals with moderate to high heterozygosity for any of the studied species, and neither are they adequately representing the species' potential climatic refugia. They also have poor coverage of the distinct adaptive and neutral genetic clusters that comprise each of the species' distributions. By incorporating the evolutionary attributes into the prioritization process, we identified priority areas that would address all of the shortfalls for only a minor increase in the size of the protected area system. In comparison, the prioritization generated following conventional approaches, despite encompassing a similar extent, did not substantially improve representation of the species' evolutionary attributes.

4. *Synthesis and applications.* We introduce a framework for incorporating adaptive and neutral evolutionary processes into conservation planning. This framework can reveal weaknesses in the coverage of climatic refugia, genetic diversity and

potential local adaptations by existing protected areas. Moreover, it can identify priority areas to improve conservation of evolutionary processes. Since neglecting evolutionary processes can impair conservation plans, we recommend using evolutionary data to inform decision-making where possible.

KEYWORDS

adaptation, climate change, evolution, genetic diversity, prioritization, protected areas, resilience, single-nucleotide polymorphism

1 | INTRODUCTION

Protected areas are essential to help safeguard biodiversity from anthropogenic pressures (Watson, Dudley, Segan, & Hockings, 2014). Since resources are limited, systematic analyses are used to reveal shortfalls in existing protected area systems (termed gap analyses) and develop cost-effective plans for expanding them (termed prioritizations; Margules & Pressey, 2000). Protected area systems can promote the long-term persistence of species by securing habitats and populations that are important for maintaining their evolutionary processes (Moritz, 2002). Specifically, neutral evolutionary processes create and regulate genetic diversity, and adaptive evolutionary processes enable species to overcome changes to their surrounding environments (reviewed in Funk, McKay, Hohenlohe, & Allendorf, 2012). As a consequence, there has been increasing interest in incorporating evolutionary processes into conservation planning (Carvalho, Brito, Crespo, & Possingham, 2011; Carvalho et al., 2017; Hanson, Rhodes, Possingham, & Fuller, 2018; Nielsen, Beger, Henriques, Selkoe, & von der Heyden, 2017; Paz-Vinas et al., 2018; Thomassen et al., 2011).

Protected areas should ideally maintain and enhance adaptive and neutral evolutionary processes (Sgro, Lowe, & Hoffmann, 2011). Previous prioritizations have incorporated such processes at the intraspecific level using maps of related attributes (hereafter, evolutionary attributes). For example, maps of neutral genetic diversity and gene flow have served as evolutionary attributes for neutral processes (Hanson, Fuller, & Rhodes, 2019; Hanson et al., 2018; Nielsen et al., 2017). On the other hand, maps of climatic refugia, environmental conditions, morphological variation and putatively adaptive genetic diversity have served as evolutionary attributes for adaptive processes (Cowling, Pressey, Rouget, & Lombard, 2003; Game, Lipsett-Moore, Saxon, Peterson, & Sheppard, 2011; Hanson, Rhodes, Riginos, & Fuller, 2017; Thomassen et al., 2011). Although an optimally sited prioritization would represent a comprehensive range of these evolutionary attributes for each species of interest (Beger et al., 2014), long-standing challenges in mapping and operationalizing evolutionary processes have severely limited the scope of previous prioritizations.

Here, we introduce a new framework for incorporating evolutionary processes into conservation planning using three amphibian species native to the Iberian Peninsula (*Hyla molleri* Bedriaga, 1889; *Pelobates cultripes* (Cuvier, 1829) and *Rana iberica*, Boulenger, 1879). By mobilizing environmental, genetic and occurrence data, we mapped a comprehensive range of evolutionary attributes to delineate places containing (neutral attributes) individuals with moderate to high heterozygosity, different neutral genetic clusters, (adaptive attributes) different adaptive genetic clusters and climatic refugia. We then overlaid these maps with boundaries of existing protected areas to quantify shortfalls and identified priority areas to address them. We also compared these priority areas with those from conventional prioritization approaches that neglect evolutionary processes. Although challenges remain in implementing effective conservation policies, recent work—within our study area—shows how engaging with local stakeholders and governmental agencies can make systematic conservation planning exercises more policy-relevant (Pinto et al., 2019).

2 | MATERIALS AND METHODS

2.1 | Study area and species

Our study area encompassed the Iberian Peninsula (Figure 1). We examined three amphibian species with different ecological niches and natural histories that are endemic—or very nearly—to this area: the Iberian tree frog (*H. molleri*), western spadefoot toad (*P. cultripes*) and Iberian frog (*R. iberica*; Loureiro, Ferrand de Almeida, Carretero, & Paulo, 2008; Pleguezuelos, Márquez, & Lizana, 2002). *Hyla molleri* inhabits forests, grasslands and shrublands throughout south-western France and most of the Iberian Peninsula, excepting eastern and southern Spain, and reproduces in water bodies with abundant vegetation. *Pelobates cultripes* occurs across the Mediterranean bioregion of the Iberian Peninsula, south-eastern and south-western France, with scattered populations in the Atlantic bioregion of north-western Iberia. It prefers soft sandy soils for burrowing, and reproduces in stagnant water bodies. *Rana iberica* occurs across northern and north-western Iberia, and the Sistema Central mountains in central Iberia. It inhabits streams and rivers with riparian vegetation, and small shallow water bodies. All of these species have spatially structured patterns of neutral genetic variation (Gutiérrez-Rodríguez, Barbosa, & Martínez-Solano, 2017; Sánchez-Montes, Recuero, Barbosa, & Martínez-Solano, 2019; Teixeira, Gonçalves, Ferrand, García-París, & Recuero, 2018). Furthermore, *R. iberica* and *P. cultripes* are Near Threatened on the Red List by the International Union for
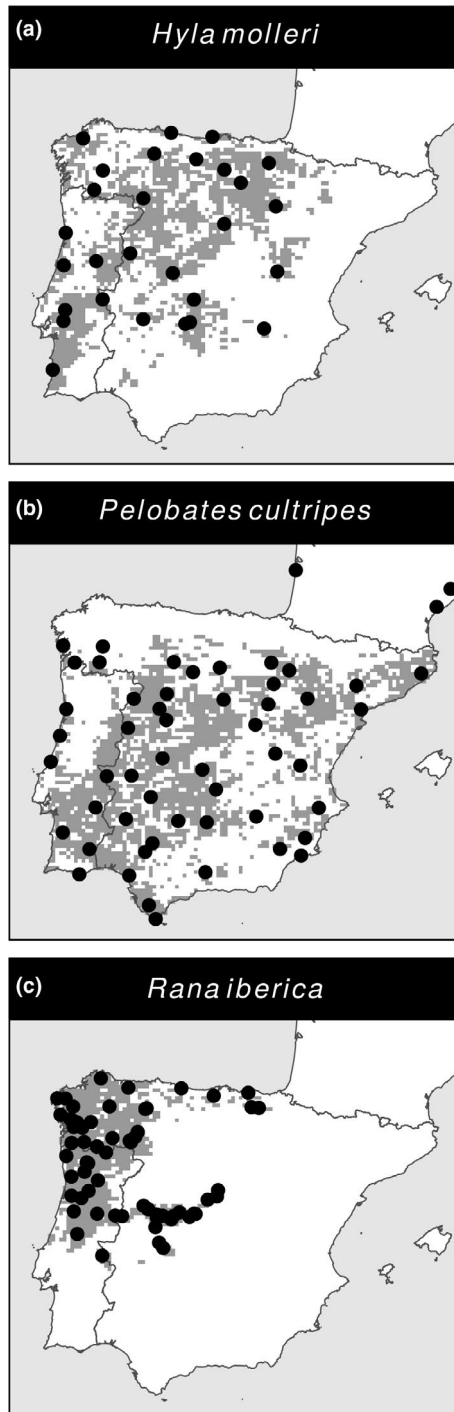
FIGURE 1 Panels (a–c) show maps delineating the (dark grey) spatial distribution of each study species within the study area and (points) sampling sites

Conservation of Nature, requiring site management and protection (IUCN, 2019).

2.2 | Data

Tail and toe biopsy samples were collected from tadpoles and adults (respectively) from multiple sites within climatically distinct zones

inside each species' distribution across the Iberian Peninsula and south-eastern France (number of samples for *H. molleri*: 92, *P. cultripes*: 184 and *R. iberica*: 194; Figure 1). Additional samples were obtained from museum collections (Gutiérrez-Rodríguez et al., 2017). ExtractMe Genomic DNA 96-Well (DNA GDAŃSK) and QIAamp DNA Micro (QIAGEN GmbtH) kits were used to extract genomic DNA. Depending on the amount of starting material, DNA samples were then concentrated with vacuum centrifugation and standardized to 300–500 ng per sample. These samples were genotyped by Diversity Arrays Technology Pty. Ltd, following the standard DArTSeq protocol (Sansaloni et al., 2011), using single-nucleotide polymorphisms (SNPs). The genomic datasets were then filtered to exclude unreliable data (see Appendix S1, Table S1, retaining a subset of samples and loci for *H. molleri*: 91 and 11,390, *P. cultripes*: 178 and 25,681, and *R. iberica*: 169 and 24,458 respectively; 1–8 samples per locality per species). We excluded three *P. cultripes* samples (not reported in previous statistics) because they were determined to have incorrect sampling locality information after cross-referencing preliminary results with previous work (Gutiérrez-Rodríguez et al., 2017). The majority of processing was completed using the R statistical computing environment (version 3.5.3; R Core Team, 2019). Genetic data were processed using PGDSpider (version 2.1.1.5) and the dartR R package (Gruber & Georges, 2018; Lischer & Excoffier, 2012).

We compiled datasets to map evolutionary attributes for each species. To standardize spatial analyses and define the planning units for assessments and prioritizations, we created a grid with 10 × 10 km cells covering the study area (UTM Zone 30N). Species distribution data were obtained from national atlases (10 × 10 km resolution; Figure 1; Loureiro et al., 2008; Pleguezuelos et al., 2002). Seven bioclimatic variables were obtained to characterize contemporary climate regimes (1950–1990; BIO1: annual mean temperature, BIO2: mean diurnal range, BIO3: isothermality, BIO8: mean temperature of wettest quarter, BIO9: mean temperature of driest quarter, BIO13: precipitation of wettest month and BIO14: precipitation of driest month; 2.5′ resolution; Hijmans, Cameron, Parra, Jones, & Jarvis, 2005). These variables were selected from the 19 available bioclimatic variables to avoid multicollinearity issues (following Naimi, Hamm, Groen, Skidmore, & Toxopeus, 2014). Projections of the seven bioclimatic variables from 19 general circulation models were also obtained to characterize plausible future climatic regimes (2070), assuming representative concentration pathway 4.5 (2.5′ resolution; Hijmans et al., 2005). Additionally, soil bedrock data were obtained to characterize abiotic conditions influencing vegetation communities (1 km² resolution; Panagos, 2006; Van Liedekerke, Jones, & Panagos, 2006). Since 3.94% of the planning units (grid cells) were missing bedrock classification data, missing data were interpolated using modal values of adjacent locations. The datasets were then reprojected and aligned to the spatial grid (Figures S1–S20). Spatial processing was performed using the sf and raster R packages (Hijmans, 2018; Pebesma, 2018).

We also compiled datasets to assess existing protected areas and generate prioritizations. Data delineating the boundaries of protected

areas in the study area were also obtained (IUCN & UNEP-WCMC, 2018) and cleaned following standard practices using the `wdpar` R package (Appendix S2; Hanson, 2019). Additionally, 1,574 Sites of Community Importance (Habitat Directive), 709 Special Protection Areas (Birds Directive) and 285 Natura 2000 sites were excluded because such places are not strictly managed as wildlife refugia (e.g. hunting is permitted; DG XI.D.2, 1998). The most recent version of the human footprint index, describing anthropogenic pressure during 2009, was obtained to characterize opportunity costs for establishing new reserves (1 km^2 resolution; Venter et al., 2016, 2018). Since 1.23% of the study area were missing human footprint data, an inverse distance weighting interpolation procedure was used to predict missing values. These datasets were then reprojected and aligned to the spatial grid, and grid cells with over 50% coverage by protected areas were subsequently treated as protected (Figures S21 and S22).

2.3 | Statistical analysis

Many species comprise multiple distinct evolutionary units (Funk et al., 2012). To account for such units, we grouped the individuals sampled for each species into broad-scale genetic clusters (hereafter, genetic lineages) using `Structure` (version 2.3.4; Pritchard, Stephens, & Donnelly, 2000), `clumpp` (version 1.1.2; Jakobsson & Rosenberg, 2007) and Evanno's ΔK method (Evanno, Regnaut, & Goudet, 2005) implemented in the `pophelper` R package (Appendix S3 and Figures S23–S24; Francis, 2017). We also verified these analyses using the `tess3r` R package (Figure S25; Caye, Jay, Michel, & François, 2018). Next, we used phylogeographic interpolation to predict the spatial distribution of each genetic lineage—per the `Structure` analyses—for each species using the `phylin` R package (Tarroso, Carvalho, & Velo-Antón, 2019). For each species, semi-variograms were estimated using exponential, Gaussian, linear and spherical models fitted with a maximum of 100,000 iterations and evaluated using coefficient of determination statistics (Table S2). The best semi-variogram fit for each species was then used with ordinary kriging to predict the spatial distribution of each genetic lineage for each species (Figures S26–S27).

We identified loci potentially under selection using outlier loci detection and environmental association analyses (Appendix S4). This step was completed after grouping the samples into genetic lineages because one of these analyses requires such information. To prevent individuals of uncertain ancestry from biasing these analyses, they were excluded (i.e. individuals with a maximum membership probability below 75%). The following environmental association analyses were performed using the contemporary climate data: latent factor mixed models (using the `lfmm` R package; Caye, Jumentier, Lepeule, & François, 2019), and redundancy analyses (Forester, Lasky, Wagner, & Urban, 2018). The following outlier detection analyses were also performed: multinomial-Dirichlet models (via BayeScan version 2.1, and the `qvalue` R package; Foll & Gaggiotti, 2008; Storey, Bass, Dabney, & Robinson, 2015), Moran

spectral outlier detection analyses (using the `spdep` R package; Bivand, Pebesma, Gomez-Rubio, & Pebesma, 2013; Wagner, Chávez-Pesqueira, & Forester, 2017) and principal components analyses (using the pcadapt, `nFactor` R, and `qvalue` R packages; Luu, Bazin, & Blum, 2017; Raîche, 2010). After running these analyses (Figures S28–S32), loci flagged in at least two of the outlier loci analyses or two of the environmental association analyses were subsequently treated as putatively adaptive and the remainder as neutral (number of adaptive loci for *H. molleri*: 18, *P. cultripes*: 151 and *R. iberica*: 215; Table S3).

We fitted environmental niche models for each species using MaxEnt (Hirzel & Le Lay, 2008; Phillips, Dudík, & Schapire, 2017). After fitting the models using the atlas and contemporary environmental data (using the `ENMeval` R package; Muscarella et al., 2014), they were used to map contemporary habitat suitability for each species (Appendix S5, Figure S33; Table S4). They were also used to produce 19 maps of predicted future habitat suitability for each species according to the future bioclimatic projections and contemporary bedrock data (Figures S34–S36).

2.4 | Evolutionary attributes

We mapped four evolutionary attributes for each species. Specifically, two evolutionary attributes related to neutral processes (individual heterozygosity and neutral genetic clusters) and two evolutionary attributes related to adaptive processes (adaptive genetic clusters and climatic refugia). See Table 1 for rationale.

2.4.1 | Individual heterozygosity

For each species, we (a) calculated heterozygosity scores for each individual using neutral loci (using the `inbreedR` R package; Coltman, Pilkington, Smith, & Pemberton, 1999; Stoffel et al., 2016), (b) computed the average heterozygosity of each sampling locality to facilitate spatial interpolation, (c) interpolated the heterozygosity scores for each planning unit inside the species' distribution using thin plate splines (Figure S37; Table S5) and (d) for each genetic lineage, clamped values for planning units with heterozygosity scores below the median value to zero to exclude planning units with low heterozygosity (Figure S38).

2.4.2 | Neutral genetic clusters

For each genetic lineage within each species, we (a) used hierarchical genetic cluster analyses to classify individuals into fine-scale genetic clusters (hereafter, genetic clusters) using neutral loci (using Gaussian mixture model-based cluster analyses implemented in the `mclust` R package; Scrucca, Fop, Murphy, & Raftery, 2016; following Van den Wyngaert, Möst, Freimann, Ibelings, & Spaak, 2015), (b) estimated semi-variograms for

TABLE 1 Description of evolutionary attributes

Attribute	Rationale	Targets
Neutral evolutionary processes		
Individual heterozygosity	By conserving planning units that contain individuals with moderate to high heterozygosity, protected area systems may secure individuals with high genetic fitness that belong to demographically stable populations (Grueber et al., 2008)	Represent 15% of the planning units with moderate to high individual heterozygosity scores, weighted by the scores, associated with each genetic lineage in each species (Figure S38)
Neutral genetic clusters	By conserving a set of planning units that contain individuals with different combinations of alleles, protected area systems can limit erosion of overall genetic diversity (Carvalho et al., 2011; Moritz, 2002)	Represent 15% of the planning units associated with each genetic cluster in each species (Figures S39–S41)
Adaptive evolutionary processes		
Adaptive genetic clusters	By conserving a set of planning units that contain individuals with different combinations of alleles at loci that are (likely) under selection, protected area systems can prevent loss of locally adaptive genetic variants (Sgro et al., 2011)	Represent 15% of the planning units associated with each genetic cluster, weighted by the non-neutrality scores, in each species (Figures S42–S44)
Climate refugia	By conserving planning units that are expected to experience relatively slow declines in environmental suitability, protected area systems may secure places that provide more time for species to adapt to changing conditions (Game, Wallis, & Jamieson, 2011)	Represent 15% of the planning units with climatic refugia, weighted by long-term suitability scores, for each genetic lineage in each species (Figure S46)

phylogeographic interpolation following the previously described methodology (Table S6), (c) predicted the spatial distribution of each genetic cluster within the lineage (using the `phylin R` package) and (d) for each species, associated each planning unit with the most likely genetic cluster to occupy it (using cluster assignments for sampled planning units and interpolated assignments for those missing samples; Figures S39–S41). We used Gaussian mixture model-based cluster analyses since they can be applied to both neutral and adaptive loci.

2.4.3 | Adaptive genetic clusters

For each species, we employed the same methodology described above—except using putatively adaptive loci—to map genetic clusters within each genetic lineage (Table S6). To account for spatial variation in the strength of adaptive processes: for each species, we (a) performed metric multi-dimensional scaling analyses with Manhattan distances (Table S7) to produce separate ordinations for the putatively adaptive and neutral loci (using the `vegan R` package; Oksanen et al., 2018), (b) subjected the ordinations to a Procrustes analysis, (c) computed the absolute values of the residuals from the Procrustes analysis to create non-neutrality scores that assign greater values to individuals that contain combinations of alleles at putatively adaptive loci that deviate more strongly from patterns among neutral loci, (d) spatially interpolated the scores across each species' distribution using thin plate splines (Table S8) and (e) used the non-neutrality scores to weight planning units within each adaptive genetic cluster (Figures S42–S44).

2.4.4 | Climatic refugia

For each species, we combined maps of contemporary and potential future habitat suitability (Figures S33–S36) to produce a single map denoting long-term habitat suitability (Figure S45). Specifically, we used harmonic means because they penalize localities with high variation in suitability scores. Thus, places containing higher long-term habitat suitability scores are more likely to serve as future climatic refugia. For each genetic lineage, we clamped planning units with scores below the median value to zero to exclude planning units with less suitable conditions (Figure S46).

2.5 | Protected area assessments and prioritizations

We assessed how well the evolutionary attributes are covered by existing protected areas (termed representation) and compared their percent coverage to target thresholds to reveal shortfalls. Ideally, targets would be derived using mathematical models related to species' persistence. However, no such models currently exist and so, following previous work (e.g. Carvalho et al., 2011; Thomassen et al., 2011), we set fixed targets to represent 15% of each evolutionary attribute for each species (see Table 1).

We generated a prioritization using the evolutionary attributes (using the `prioritizr R` package; Hanson, Schuster, et al., 2019). To assess the relative importance of priority areas, we derived irreplaceability scores using a modified version of the replacement cost method (Appendix S6; Cabeza & Moilanen, 2006). To gain insight into their roles, we identified which evolutionary attribute was helped the most by each priority area (measured using percentage-wise increase
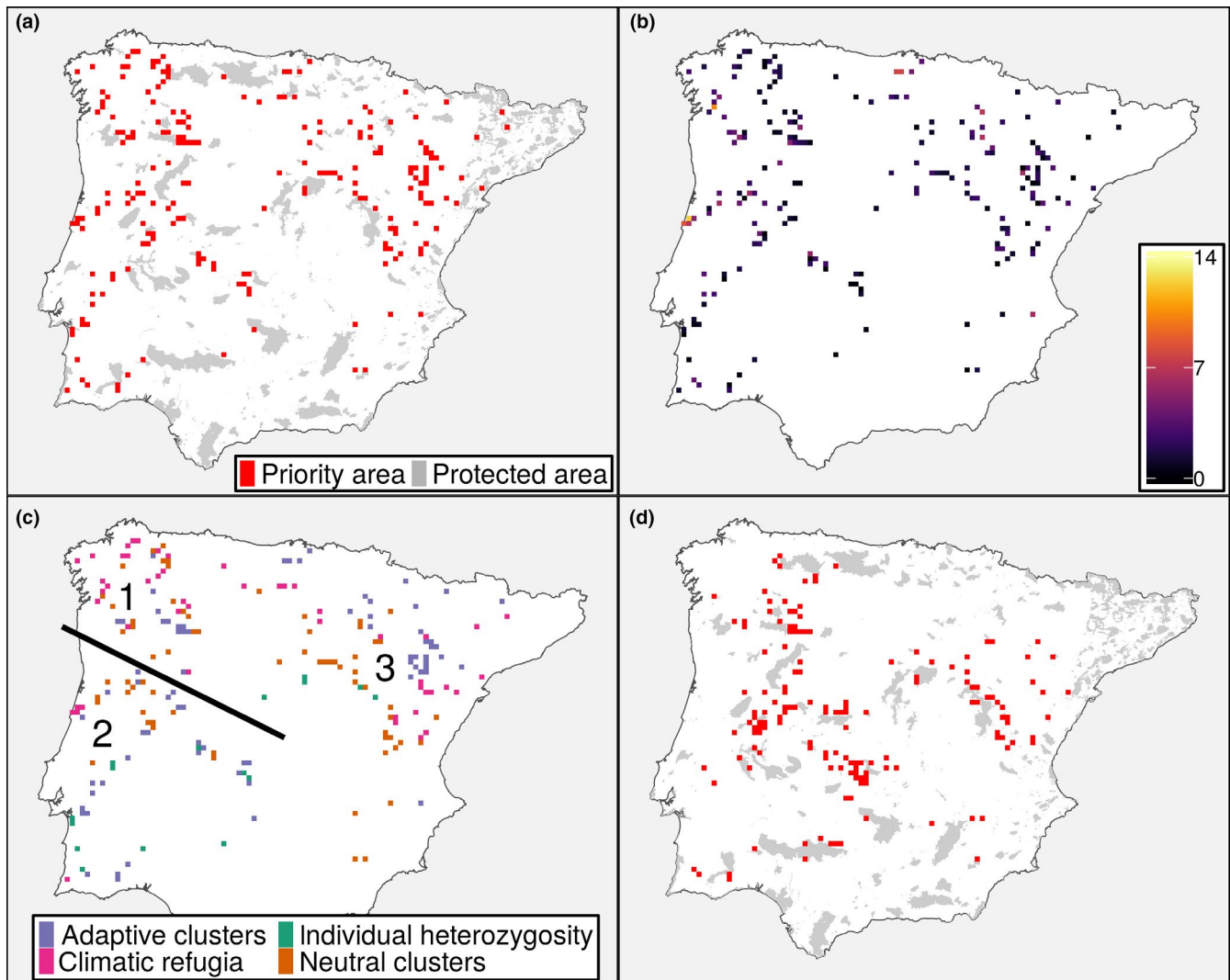
FIGURE 2 Maps show (a) priority areas for representing the species' evolutionary attributes, (b) their irreplaceability scores, (c) the primary evolutionary attribute for which they increase representation and (d) the prioritization generated following conventional approaches that neglect evolutionary processes. Labels in (c) correspond to different regions of priority areas, and the line demarcates regions 1 and 2

in representation). We visually grouped the priority areas primarily associated with the same evolutionary attributes into regions to facilitate interpretation (shown in Figure 2c). To understand how well conventional approaches represent evolutionary processes, we generated a prioritization using only the species' distribution data—omitting the evolutionary attributes—to secure 15% of each species' distribution within the study area. All prioritizations were generated using the minimum set formulation of the reserve selection problem with planning unit costs set as the human footprint index and solved to optimality (using Gurobi version 8.1.0; Gurobi Optimization, LLC, 2018).

3 | RESULTS

Existing protected areas in the Iberian peninsula are failing to adequately represent evolutionary attributes for *H. molleri*, *P. cultripes* and *R. iberica* (Table 2; Table S8). Each of these species comprise two

distinct genetic lineages. Protected areas are not adequately representing places predicted to contain individuals with moderate to high heterozygosity—none of the genetic lineages for any of the three species have adequate representation of such places. When examining fine-scale patterns of intraspecific genetic variation within each of the species' genetic lineages, less than one-third of the species' genetic clusters classified using neutral loci are adequately represented (*H. molleri*: 16.67%, *P. cultripes*: 12.5% and *R. iberica*: 25%). Additionally, none of the genetic clusters identified using putatively adaptive loci are adequately represented for *H. molleri* and *P. cultripes*, and only half of such genetic clusters are adequately represented for *R. iberica*. Furthermore, none of the genetic lineages for *H. molleri* and *P. cultripes* and only one of the two genetic lineages comprising *R. iberica* have adequately represented climatic refugia.

Priority areas were identified for improving the representation of the species' evolutionary attributes by protected areas (Figure 2a; Table 2; Table S9). If added to the existing protected area system,

TABLE 2 Performance of the existing protected area system, prioritization generated using evolutionary attributes and conventional prioritization that neglected evolutionary processes. For a given species, each evolutionary attribute is associated with multiple genetic lineages (identified using the `Structure` software) or multiple genetic clusters (identified using the `mclust R` package). Data show the number and percentage of clusters or lineages that are adequately represented by a given conservation plan for a given evolutionary attribute. Bold font indicates adequate representation

Species	Process	Attribute	Number of clusters/ lineages	Existing protected areas	Evolutionary prioritization	Conventional prioritization
Hyla molleri	Neutral	Individual heterozygosity	2	0 (0%)	**2 (100%)**	1 (50%)
		Neutral genetic clusters	6	1 (16.67%)	**6 (100%)**	3 (50%)
	Adaptive	Adaptive genetic clusters	9	0 (0%)	**9 (100%)**	3 (33.33%)
		Climatic refugia	2	0 (0%)	**2 (100%)**	1 (50%)
Pelobates cultripes	Neutral	Individual heterozygosity	2	0 (0%)	**2 (100%)**	1 (50%)
		Neutral genetic clusters	8	1 (12.5%)	**8 (100%)**	4 (50%)
	Adaptive	Adaptive genetic clusters	9	0 (0%)	**9 (100%)**	3 (33.33%)
		Climatic refugia	2	0 (0%)	**2 (100%)**	1 (50%)
Rana iberica	Neutral	Individual heterozygosity	2	0 (0%)	**2 (100%)**	0 (0%)
		Neutral genetic clusters	8	2 (25%)	**8 (100%)**	4 (50%)
	Adaptive	Adaptive genetic clusters	6	3 (50%)	**6 (100%)**	3 (50%)
		Climatic refugia	2	1 (50%)	**2 (100%)**	1 (50%)

these places would increase the size of the protected area estate from 8.65% to 12.1% of the Iberian Peninsula. Most priority areas have relatively low irreplaceability scores (<3; Figure 2b) and so they can be substituted by places with low human pressure. Many of the priority areas are sited at the intersection of the northern genetic lineages of *H. molleri* and *R. iberica* (region 1, Figure 2c). These specific locations—in addition to helping to address shortfalls for other evolutionary attributes—markedly improve the capacity of existing protected areas to represent climatic refugia and different adaptive and neutral genetic clusters. Moving south, more priority areas are sited at the intersection of the southern lineages of these two species (region 2, Figure 2c). They mainly help increase the representation of adaptive and neutral genetic clusters, and individuals with moderate to high heterozygosity. Further east, more priority areas are sited in places containing the eastern genetic lineage for *P. cultripes* (region 3, Figure 2c). They primarily help increase the representation of climatic refugia and adaptive and neutral genetic clusters (similar to region 1). These results suggest that different priority areas may be important for different evolutionary processes.

The prioritization generated using conventional approaches that neglect evolutionary processes had poor coverage of the species' evolutionary attributes (Figure 2d; Table 2; Table S10). It only adequately represented places containing individuals with moderate to high heterozygosity for half of the genetic lineages comprising *H. molleri* and *P. cultripes* and none of the genetic lineages comprising *R. iberica*. It was also only able to adequately represent half of the neutral genetic clusters associated with each of the three species. Additionally, it only adequately represented one-third of the adaptive genetic clusters for *H. molleri* and *P. cultripes* (both 33.33%), and provided no improvement for *R. iberica*. Similarly, it increased coverage to adequately represent climatic refugia for half of the genetic lineages comprising *H. molleri*

and *P. cultripes*, and provided no improvement for *R. iberica*. Despite performing much worse than the prioritization generated using evolutionary attributes, this prioritization encompassed a similar percentage of the study area (11.16%) and cost (i.e. sum human footprint index values) only 8.88% less. These findings demonstrate that prioritizations generated without explicitly considering evolutionary processes can fail to represent them.

4 | DISCUSSION

We developed a new framework for incorporating adaptive and neutral evolutionary processes into systematic conservation planning and applied it to three amphibian species (*H. molleri*, *P. cultripes* and *R. iberica*). Although existing protected areas in our study area are helping to conserve biodiversity (Araújo, Lobo, & Moreno, 2007), we found that additional protected areas are needed to help maintain adaptive and neutral evolutionary processes for these species. We reveal priority areas that would achieve adequate representation of such places for only a minor increase in the protected area system. We also show that neglecting evolutionary processes can produce prioritizations that fail to substantially improve the representation of evolutionary processes—further highlighting the importance of accounting for them (Carvalho et al., 2011; Hanson, Fuller, et al., 2019). Our work helps pave the way for the widespread integration of evolutionary data into conservation decisions (Beger et al., 2014; Carvalho et al., 2017; Cowling et al., 2003).

Our framework enables conservation planners to explicitly and comprehensively account for adaptive and neutral evolutionary processes. We built this framework on the strengths and weaknesses of

previous frameworks (e.g. Beger et al., 2014; Diniz-Filho et al., 2012; Hanson et al., 2018). It accounts for the evolutionary processes operating within each species separately (Hanson et al., 2018). Instead of relying on surrogates (Carvalho et al., 2011; Ponce-Reyes, Clegg, Carvalho, McDonald-Madden, & Possingham, 2014), it uses metrics derived from putatively adaptive and neutral loci (Hanson et al., 2017). Additionally, it uses genetic clusters—instead of allele frequencies (Diniz-Filho et al., 2012)—to characterize fine-scale patterns of genetic diversity so that spatial interpolation routines can be used to make predictions for planning units missing data (Carvalho et al., 2017). It also emphasizes places containing individuals with greater genetic diversity (Beger et al., 2014; Nielsen et al., 2017; Paz-Vinas et al., 2018; Thomassen et al., 2011). It accounts for abiotic factors that influence evolutionary processes (i.e. climatic refugia; Game et al., 2011). Furthermore, by leveraging the minimum set formulation of the reserve selection problem (Beger et al., 2014; Nielsen et al., 2017), optimal solutions can be obtained quickly for planning exercises involving thousands of planning units (unlike Hanson et al., 2018).

We found that existing protected areas in the Iberian Peninsula are not adequately representing evolutionary attributes for the three amphibian species. This result is not surprising given that the existing protected area system was not designed specifically for these species—much less their evolutionary processes. Many protected areas in Europe are in places that historically served as royal hunting grounds, gardens or forests (European Environment Agency, 2012). Additionally, they may also have been established opportunistically in places that provide limited benefits (Joppa & Pfaff, 2009). We also identified priority areas to improve the representation of the species' evolutionary attributes. Since we examined far fewer species than previous studies—and differences in the spatial patterns of intraspecific genetic diversity among species can alter priorities (Paz-Vinas et al., 2018)—few of the priority areas identified in this study coincide precisely with those in previous studies (Araújo et al., 2007; Carvalho et al., 2011, 2017). Despite such fine-scale differences, our study reinforces previous work that has identified central Portugal, and north-western (Galicia) and eastern (Aragón and Comunidad Valenciana) Spain as important conservation areas in the Iberian Peninsula (Araújo et al., 2007; Carvalho et al., 2017).

Our results show that conventional prioritization approaches that do not explicitly incorporate evolutionary attributes may fail to maintain evolutionary processes. These results contribute to the growing body of evidence indicating that neglecting evolutionary processes can undermine conservation (Carvalho et al., 2011; Hanson, Fuller, et al., 2019; Nielsen et al., 2017; Thomassen et al., 2011). For instance, within the Iberian Peninsula, prioritizations generated using only species' distribution data secure much less intraspecific neutral genetic diversity and environmental heterogeneity—a putative surrogate for adaptive genetic diversity (Hanson et al., 2017)—than prioritizations generated using these evolutionary attributes directly (Carvalho et al., 2011, 2017). Outside of the Iberian Peninsula, prioritizations generated using such conventional approaches secure much less intraspecific genetic diversity and morphological variation

(Nielsen et al., 2017; Thomassen et al., 2011; but see Hermoso et al., 2016). Furthermore, priority areas identified at the global-scale using such conventional approaches secure much less of the diversity in environmental conditions found across species' distributions (Hanson, Rhodes, et al., 2020)—meaning they could omit important places for climate change adaptation (Sgro et al., 2011).

Our study has limitations. First, while real-world planning exercises should consider far more than three species (Margules & Pressey, 2000), our main aim was to introduce the framework—not to identify priority areas for conserving biodiversity across the Iberian Peninsula. Second, one remaining challenge is identifying effective targets for evolutionary attributes that will substantially improve species' long-term persistence. Although we adopted fixed targets following previous studies (e.g. Carvalho et al., 2011), different targets will likely be needed for different evolutionary attributes (Pressey, Cowling, & Rouget, 2003). Third, although samples were collected to maximize our capacity to map adaptive genetic variation, we were unable to assess population-level genetic diversity (e.g. allelic richness). Fourth, our five analyses for detecting putatively adaptive loci did not yield highly consistent results (further explored in A. Marques, J.O. Hanson, M. Camacho-Sanchez, Í. Martínez-Solano, C. Moritz; P. Tarroso, G. Velo-Antón; A. Veríssímo; & S.B. Carvalho, unpubl. data), and so further experimentation is required. Additionally, we detected far fewer putatively adaptive loci for *H. molleri*, and so our results may not reflect its full range of adaptive genetic variation. Fifth, although we followed standard practices for modelling future species' distributions, uncertainty in future climate projections limits our ability to correctly identify climatic refugia (Game et al., 2011). Sixth, establishing protected areas alone is often insufficient, and populations may also require direct management interventions (e.g. genetic rescue; Flanagan, Forester, Latch, Aitken, & Hoban, 2018). Finally, future research could examine gene flow (e.g. using Daigle et al., 2020; Hanson, Fuller, et al., 2019).

Conservation plans need to maintain adaptive and neutral evolutionary processes to maximize the persistence of biodiversity. Since challenges remain in explicitly using these processes to inform decision-making, we developed a framework to identify priority areas using ecological and evolutionary data. Further research is needed to inform setting representation targets for evolutionary processes. In the meantime, we advise conservation scientists and practitioners to carefully consider using evolutionary processes to guide reserve selection.

AUTHORS' CONTRIBUTIONS

J.O.H. and S.B.C. conceived the study; Í.M.-S., G.V.-A. and S.B.C. conducted the fieldwork; A.M. performed the lab work; J.O.H., M.C.-S., A.M., G.V.-A., A.V. and S.B.C. developed the methodology; J.O.H. analysed the data and led the writing of the manuscript. All authors contributed critically to writing the manuscript, interpreting the results, and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Code and data—excepting the World Database on Protected Areas (IUCN & UNEP-WCMC, 2018), ESDBv2 Raster Library (Panagos, 2006; Van Liedekerke et al., 2006), and atlas data (Loureiro et al., 2008; Pleguezuelos et al., 2002) which are previously published datasets—available via the Zenodo Digital Repository https://doi.org/10.5281/zenodo.3752249 (Hanson, Marques, et al., 2020).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.